(Bureau of Agricultural & Industrial Chemistry - Western Region)

FOR 1953-54 PROGRESS REPORTS FOR

CITRUS FRUIT ADVISORY COMMITTEE

UTILIZATION RESEARCH

PROGRESS ON WORK UNDER WAY

Composition and Quality Evaluation and Preservation

ORANGE

Identification of Nitrogenous Constituents in Orange Juice Products — BAIC

It was reported previously by the Pasadena Laboratory that California Valencia orange juice contains nine major nitrogenous constituents including alanine, arginine, aspartic acid, asparagine, gamma-aminobutyric acid, glutamic acid, glutamine, proline and serine, as well as three minor nitrogenous constituents, histidine, cysteine and glutathione. These compounds contain essentially all of the nitrogen in filtered orange juice and constitute approximately 5-10 percent of the total orange juice solids.

The qualitative studies on the nitrogenous constituents in citrus juices have been extended by the development of a rapid small-scale paper chromatography procedure for the quantitative estimation of free amino acids in orange juice. Quantitative estimations of five amino acids (aspartic acid, glutamic acid, alanine, serine and gamma-aminobutyric acid) in fresh and heated (12 minutes at 100° F.) canned, single strength orange juice indicated that 20 percent of the aspartic

acid and serine and 50 percent of the alanine and glutamic acid, but none of the gamma-aminobutyric acid, were destroyed during the heat treatment. No further changes were observed in the heated juice after storage for two months at 100° F.

The same five amino acids were estimated in the juices obtained from California Valencia oranges harvested at various stages of maturity during each of the 1950, 1951 and 1952 seasons. The data obtained confirmed previous preliminary observations which showed wide, regular variations in the amount of amino acids, especially gamma-aminobutyric acid, present in the juice of fruit harvested at different stages of maturity.

The ratio of gamma-aminobutyric acid:acid appears to be a more sensitive index of maturity and organoleptic quality than the currently employed Brix:acid maturity index. The validity of this potential maturity index will be further explored during the coming year and the quantitative estimations of the other amino acids in orange juice will be continued at the present rate.

(See Proposals for Committee Consideration)

Publications: Nitrogenous Constituents in Citrus Fruits, I. Some Free Amino Acids in Citrus Juices Determined by Small-Scale Filter-Paper Chromatography, J. C. Underwood and L. B. Rockland, Food Res. 18, 17 (1953); Studies on Small-Scale Filter-Paper Chromatography. I. Factors Affecting the Separation and Sequence of Amino Acids, L. B. Rockland and J. C. Underwood, Abstracts of Papers, 14B, 123rd Meeting of the American Chemical Society, Los Angeles, California, March 1953.

"Studies on Small-Scale Filter-Paper Chromatography. II. A Rapid Two-Dimensional Procedure," L. B. Rockland and J. C. Underwood, Abstracts of Papers, 15B, 123rd Meeting of the American Chemical Society, Los Angeles, California, March, 1953.

Identification of Volatile Flavoring Constituents in Canned Orange Juices — BAIC

In carrying on the work of isolation and identification of the volatile constituents of citrus juices at the Pasadena Laboratory, which began with the grapefruit constituent studies, 2,500 gallons each of fresh, freshly canned, and stored canned orange juices have been processed to recover the volatile flavoring constituents for identification studies. The compositional analysis of the water-soluble volatile constituents obtained in this work has been previously reported (1952).

Separation and identification of the water-insoluble volatile oil fraction is now being carried out using the newly developed chromato-strip technique, and the volatile oils have been separated into two major fractions--hydrocarbon constituents and oxygenated constituents.

The major components of the hydrocarbon constituents has been identified as limonene. Two additional hydrocarbon constituents have been identified in this fraction; and two sesquiterpenes and a trace amount of an additional hydrocarbon have been isolated but not yet positively identified.

The oxygenated constituents of the fresh and of the stored canned juice have been separated into individual components. There is a total of approximately twenty-two individual oxygenated constituents.

Seven of the constituents found in the fresh juice have already been identified as well as four of the constituents found in the stored canned juice.

Since some of the oxygenated constituents occur in extremely minute amounts, it has become desirable to obtain larger quantities of the volatile oil fractions to complete the identification studies. These additional orange juice volatiles are now being recovered from the vacuum system of a commercial low-temperature orange juice concentration plant in California.

(See Proposals for Committee Consideration)

Publications: Chromatostrips for Identifying Constituents of Essential Oils, John M. Miller and J. G. Kirchner, Anal. Chem. 25, 1107 (1953).

Identification of Carotenoid Pigments in Orange Juice — BAIC

The work on isolation and identification of carotenoid pigments of orange juice has been continued as a part of a program of investigations at the Western Regional Research Laboratory to determine the causes of off-flavor development in canned or powdered citrus juices. Twenty-one different carotenoids have now been separated from California Valencia juice by chromatography and countercurrent distribution. Most of them have been identified, at least tentatively, and several appear to be substances not found or described elsewhere. Some of the carotenoids are present in the juice, both in the free state and also

in combination with acids (as esters). A major part of the juice carotenoids was shown to consist of epoxides which are less stable than simpler carotenoids and may contribute significantly to the development of off-flavor in canned or dehydrated juice products. Early season Valencia oranges were found to contain at least one carotenoid constituent (not yet identified) that was not found in late season fruit. More data are needed to verify this observation and to determine whether this is one of the seasonal characteristics of the fruit.

A comparison of the carotenoids of the peel and of the pulp showed them to be qualitatively similar but quantitatively different. For example, the peel was noteworthy in containing a much greater percentage of violaxanthin. This may reflect itself in commercial juicing operations where some peel oil with dissolved carotenoids may be introduced into the juice affecting its color, flavor and stability.

In future work it is planned to study changes in the amounts and composition of the carotenoids, as a means for developing improved processing procedures, that occur in storage of single strength canned orange juice and of orange powder.

Publications: Application of Countercurrent Distribution to Valencia Orange Juice Carotenoids, A. Laurence Curl, J. Agr. Food Chem., 1, 456 (1953).

Time-temperature Storage Studies on Frozen Orange Concentrates — BAIC

Time-temperature storage studies have been completed at the Pasadena Laboratory on two additional lots of commercial frozen orange

concentrate. One lot of concentrate had been partially stabilized by heating, while the other was unheated. As was observed during the previous year's work, the unheated concentrate showed relatively severe loss of cloud during cycles of varying temperatures (up to 40° F.) such as might be expected in today's commercial handling of these products. In contrast, the heat-stabilized concentrate showed but a slight loss of cloud under similar conditions. However, the effects of elevated temperatures on cloud loss were found to cumulate in the heat-stabilized concentrate, and the cloud became increasingly lower after a time-temperature history equivalent to 6-7 days at 40° F. As previously found with unheated concentrates, cloud loss in heat-treated concentrate became objectionable before any flavor change was detected. Flavor changes in both lots were observed only at the end of the 8 months' storage period, and only under the most extreme temperature histories which included two weeks' exposure to 20° F. and two weeks at 40° F. From these preliminary studies it would appear that the main advantage to heat stabilizing orange concentrate is to effect a longer retention of cloud.

An important new finding is that the effect of unfoavorable temperature periods upon flavor is also cumulative. As a result, although the concentrate may not show an immediate flavor change upon exposure to elevated temperatures, latent changes have occurred which cumulate each time the sample is subjected to an unfavorable temperature period until a detectable flavor change finally occurs. This effect is shown in that the largest percentage of samples which show flavor damage, as a result of a given temperature abuse, always comes from those

samples which in a previous cycle were exposed to the highest temperature histories. Samples exposed to less severe temperature conditions in previous cycles have a significantly smaller probability of acquiring flavor damage as a result of additional temperature abuse.

Studies were included on changes in the pectic substances occurring in frozen orange concentrates during time-temperature storage histories, and on the relationship of these changes to loss of cloud. It was observed that initial cloud loss, which occurred at 40° F., could be correlated with enzyme-catalyzed changes in the pectic substances. However, additional cloud loss was observed beyond this point which could not be directly correlated with pectic changes or with the enzyme pectinesterase (the enzyme thought to be responsible for cloud loss).

To summarize, the time-temperature tolerance studies on additional lots of frozen orange juice concentrates have shown that cloud loss, a cumulative effect, was observed prior to flavor change in both an unheated concentrate and one partially stabilized by heating. Subsequent flavor change was also cumulative. The study on orange juice concentrate will be continued at its present level in order to make it more comprehensive and to include the effects produced by varying degrees and methods of heat treatment employed for stabilization.

Microbiology of Frozen Orange Concentrates — BAIC

Investigations of the sources of contamination, incidence and sanitary significance of coliform organisms, particularly Escherichia coli, found in unpasteurized citrus juices have been continued during the past year at the Pasadena Laboratory.

As suggested in last year's report, bacteriological investigations of wash tank and borax tank solutions have shown that these may be a potent source of coliforms which sometimes find their way into frozen concentrated orange juice. Samples from tanks in 28 out of 47 packing houses were found to contain bacteria of the coli-aerogenes group. E. coli was found in 21 of the 28 samples containing coliforms. The E. coli level varied from less than one to 39 viable cells per ml. In 11 of the 21 E. coli-bearing solutions, the organism was present at a level of 10 or more per ml. At this level it is possible that each orange could be inoculated while passing through the bath. In comparing different methods now used for fruit cleaning, it was found that the hypochlorite method is the best for eliminating coliforms; no coliforms were recovered from solutions taken from the tanks in six houses using the Hypochlor method for fruit washing.

The comparison of boric acid broth and lactose broth as presumptive media for the enrichment of E. coli from citrus juices has been concluded. Over 400 samplings of juice and fruit rinsings were examined, and these were inoculated into 3,450 tubes of each medium for comparison. Results indicated the boric acid broth was the superior medium since the number of false positive tests was reduced by 90 percent while E. coli was recovered 1.6 times more often than with the lactose broth.

The direct microscopic examination of citrus juices has been investigated, and it was found that existing methods could be improved by altering the staining technique. The use of aniline oil, methylene blue and basic fuchsin combined in the proper proportions, and adjustment

of the juice to a more favorable reaction for these stains resulted in preparations in which the microbial cells are easier to detect microscopically. Since actively growing cells will reduce methylene blue it is possible to differentiate dead cells from the actively metabolizing cells. Dead or inactive cells stain a deep blue, while active cells having reduced the methylene blue will often appear as pale blue and purple bodies. This improved staining method has application in revealing the original microbial condition of frozen citrus juices and concentrates which have undergone prolonged freezing storage, and also as a rapid method for checking the sanitary conditions of processing equipment.

Accordingly, wash tanks and borax tank solutions may be a potent source of coliforms present in frozen orange juice concentrates. The hypochlorite method is the best for eliminating coliforms. This work will be continued at its present level during the coming year in order to determine, if possible, the sanitary significance of these organisms in concentrates. Studies of methods for the direct estimation of microorganisms present in citrus juices will be continued, particularly with a view to developing methods applicable to routine control in processing plants.

(See Proposals for Committee Consideration)

Publications: Comparison of Boric Acid and Lactose Broths for the Isolation of Escherichia coli from Citrus Products, E. R. Wolford, Bacteriological Proceedings, 1953, p. 24.

Effect of Pesticides on Flavor of Orange Juice Products BAIC

One year's work has been completed on this cooperative project of the Pasadena Laboratory with the Department of Entomology of the University of California Citrus Experiment Station at Riverside, California. Fruit sprayed with the three pesticides--Sulphenone, EPN and Systox--and unsprayed control fruit were processed by the Pasadena Laboratory into frozen concentrated orange juice, single-strength frozen orange juice and pasteurized single-strength orange juice. It was previously reported that no off-flavors were detected in any of the products immediately after processing.

One year's storage has now been completed on these three juice products during which they were taste-tested at three-month intervals by both the Pasadena and the Riverside groups to determine if off-flavors resulting from pesticide treatment would develop on storage. No off-flavors were detected in any of the samples by either group.

One new pesticide (amiticide, D-228) is being tested during the current year. Sprayed and unsprayed fruit has been processed into frozen concentrated orange juice, and pasteurized single strength orange juice and the storage studies including organoleptic testing are under way. No other pesticides will be tested during the present year.

(See Proposals for Committee Consideration)

Toxicity of 2-Aminopyridine BAIC

The Bureau of Plant Industry, Soils and Agricultural Engineering found that 2-aminopyridine is effective against stem-end rot in oranges.

Upon the request of that Bureau, chronic toxicity studies were undertaken at the Western Regional Research Laboratory to determine the safety of using this compound. The long-term feeding experiments on albino rats eating an adequate diet containing various dosage levels of 2-aminopyridine have been completed.

All the experimental animals have been autopsied and stained tissues of the various organs have been submitted to the pathologist for histopathological examination. Conclusions as to the safety of using this compound must await the pathologist's report.

GRAPEFRUIT

Identification of Nitrogenous Constituents in Grapefruit Juice Products — BAIC

No report for the current year. Continuation of these studies at the Pasadena Laboratory has been delayed in order to complete the quantitative studies on nitrogenous constituents in orange juice products, but will be continued during the coming year.

Publications: Nitrogenous Constituents in Citrus Fruits. I. Some Free Amino Acids in Citrus Juices Determined by Small-Scale Filter-Paper Chromatography, J. C. Underwood and L. B. Rockland, Food Research 18, 17 (1953); Studies on Small-Scale Filter-Paper Chromatography. I. Factors Affecting the Separation and Sequence of Amino Acids. L. B. Rockland and J. C. Underwood. Abstracts of Papers, 14B, 123rd Meeting of the American Chemical Society, Los Angeles, California, March 1953; Studies on Small-Scale Filter-Paper Chromatography. II. A Rapid Two-Dimensional Procedure, L. B. Rockland and J. C. Underwood,

Abstracts of Papers, 15B, 123rd Meeting of the American Chemical Society, Los Angeles, California, March 1953.

Identification of Volatile Flavoring Constituents in Canned Grapefruit Juices — BAIC

Work on the isolation and identification of the volatile flavoring constituents from 2500 gallons of each of fresh, freshly-canned, and store-canned grapefruit juice has been completed at the Pasadena Laboratory.

There was little difference in the composition of the volatile water-soluble materials of the fresh and freshly-canned juices. The freshly-canned juice contained a small amount of volatile acids and a trace of furfural which were not found in the fresh juice. The stored juice had a considerable increase in the volatile acids, methyl alcohol, and furfural over the other two. The methyl alcohol probably came from the breakdown of pectin and the furfural from the carbohydrates. The main acid found was acetic acid, with traces of several unsaturated acids. Of these water-soluble materials only furfural contributed to the development of off-flavors.

From the oil fractions of these juices, six hydrocarbons were isolated and identified. The main hydrocarbon, limonene, was found to play an important role in off-flavor development by conversion to alpha-terpineol and linalool monoxide during storage of the canned juice.

The non-hydrocarbon volatile oil is responsible for the typical grapefruit flavor of the fresh juice. However, in the freshly-canned and stored-canned juices, this particular fraction also contains the

off-flavor components of alpha-terpineol and linalool monoxide that had formed from the limonene.

A total of 30 compounds were found in the three juices examined. Of these 21 have been positively identified as known compounds, five have been established as new compounds not described in the literature, and one has been only tentatively identified. Empirical formulas were established for two unsaturated acids, and a trace of a third acid was isolated.

Among the oil materials, geraniol, N-methyl methylanthranilate, a $C_{15}H_{28}$ hydrocarbon, and a nitrogen compound $C_{13}H_{15}N$ were present only in the fresh and the freshly-canned juices. The leaf alcohol 3-hexene-1-ol was found only in the fresh juice. These five compounds were present in too small an amount to appreciably affect the flavor. The fresh-canned and the stored-canned juices were qualitatively the same, but a greater amount of these constituents were present in the stored juice. The most significant changes produced by storage were the decrease in limonene and a corresponding increase in alpha-terpineol and linalool monoxide. These latter two compounds and the furfural gave very undesirable flavors when added to fresh grapefruit juice.

The following compounds were found in the three juices: Limonene, alpha-pinene, alpha-caryophyllene, beta-caryophyllene, a $C_{15}H_{24}$ hydrocarbon, a $C_{15}H_{28}$ hydrocarbon, N-methyl methylanthranilate, caryophyllene oxide, citral, carvone, a nitrogen compound $C_{13}H_{15}N$, linalool, a compound $C_{15}H_{26}O$, carveol, a compound $C_{15}H_{22}O$, alpha-terpineol, 3-hexene-1-ol, geraniol, linalool monoxide, a compound $C_{12}H_{20}O_2$,

acetaldehyde, acetone, furfural, ethyl alcohol, methyl alcohol, acetic acid, an acid $C_6H_8O_2$, a second acid of formula $C_6H_8O_2$, traces of an additional acid, and hydrogen sulfide.

The work under this project has been essentially completed and accordingly will be discontinued.

Publications: Volatile Water-Soluble Constituents of Grapefruit Juice, J. G. Kirchner, J. M. Miller, R. G. Rice, G. J. Keller and Margaret M. Fox, J. of Agr. and Food Chem., 1, 510 (1953); Volatile Oil Constituents of Grapefruit Juice, J. G. Kirchner and John M. Miller, J. of Agr. and Food Chem. 1, 512 (1953); Chromatostrips for Identifying Constituents of Essential Oils, J. M. Miller and J. G. Kirchner, Anal. Chem. 25, 1107 (1953).

LEMON

Identification of Nitrogenous Constituents in Lemon Juice Products — BAIC

Lemon juices, like other citrus juices, are known to develop off-flavors, odors and colors during processing and storage. However, lemon juice undergoes darkening more rapidly and to a greater extent than most other processed citrus juices.

Using small-scale filter-paper chromatography techniques, ten nitrogenous compounds (amino acids) have been provisionally identified in lemon juices by the Pasadena Laboratory. Since amino acids and related nitrogenous constituents are capable of reacting with sugars to produce dark colored reaction products and objectionable odors and flavors, the possibility is being studied that their presence in lemon

juices may contribute to the "browning" and off-flavor development which may occur during storage.

Samples of fresh and pasteurized single-strength and concentrated lemon juice, with and without sulfur dioxide added, are being stored at 0° F. and 75° F. for periods up to two years. Changes in the nitrogenous constituents in the fresh and processed lemon juices before and during storage will be estimated by quantitative paper chromatography to determine further the significance of these constituents in darkening and off-flavor development in lemon juice. This work will afford sound basis for improving processing procedures for these products.

(See Proposals for Committee Consideration)

Publications: Nitrogenous Constituents in Citrus Fruits. I. Some Free Amino Acids in Citrus Juices Determined by Small-Scale Filter-Paper Chromatography, J. C. Underwood and L. B. Rockland, Food Res. 18, 17 (1953); Studies on Small-Scale Filter-Paper Chromatography. I. Factors Affecting the Separation and Sequence of Amino Acids, L. B. Rockland and J. C. Underwood, Abstracts of Papers, 14B, 123rd Meeting of the American Chemical Society, Los Angeles, California, March 1953; Studies on Small-Scale Filter-Paper Chromatography. II. A Rapid Two-Dimensional Procedure. L. B. Rockland and J. C. Underwood, Abstracts of Papers, 15B, 123rd Meeting of the American Chemical Society, Los Angeles, California, March 1953.

Identification of Flavonoid Constituents in Lemon Juice Products — BAIC

The darkening of lemon products stored at room temperature is one of the principal problems of the lemon processing industry. Although

the darkening of lime juice has been attributed to chemical reactions involving flavonoid compounds, the possible role of these compounds in the browning of lemon juice has not been previously explored.

Procedures have been developed at the Pasadena Laboratory for the extraction of flavonoid compounds from the juice and tissues of fresh lemons. These extracts have been partially fractionated by columnar chromatography into nine bands, at least one of which darkened measurably within two hours at room temperature. This fraction gave positive tests for flavonoid compounds. Flavonoids detected by filter paper and columnar chromatography in the juice, endocarp, albedo and flavedo of lemons appear to be directly related to the darkening phenomenon.

Work has been continued on the development of rapid and specific procedures for the qualitative analysis of flavonoid compounds on filter paper chromatograms as an aid to the identification of fractions obtained from chromatographic columns.

Procedures are being developed for the large-scale separation of lemon flavonoid compounds so that sufficient material will be available for chemical analyses.

Samples of fresh and pasteurized single-strength and concentrated lemon juices, with and without added sulfur dioxide, have been stored at 0° F. and 75° F. for periods up to two years. Darkening of both single-strength and concentrated juices, especially the latter, progressed very rapidly at room temperature in the absence of sulfur dioxide and were brown-black in color within one year. Samples

containing sulfur dioxide darkened significantly but less rapidly than the untreated samples. All juices stored at 0° F. maintained their original, natural color.

Chromatographic analyses of these samples will be performed during the next year.

UTILIZATION RESEARCH

PROPOSALS FOR COMMITTEE CONSIDERATION

Composition and Quality Evaluation and Preservation

Absorption of Biphenyl by Citrus Fruits During Storage and Shipping. Cooperative work should be initiated on the effects of chemical treatments for the control of decay in citrus fruits in storage and transit with particular emphasis on biphenyl. The work should include the testing of more chemicals on all kinds of citrus including a wider range of products, containers and storage conditions. Studies should be made of the effects of decay-inhibiting chemicals on changes in chemical composition and of citrus fruits, and on the quality of products made from such treated fruits,. Work on the development of methods for measuring the quantities of these chemicals absorbed by citrus fruits, and appearing in products made from treated citrus fruits, needs to be done.

Toxicity of Fungicides. It is recommended that the toxicity of biphenyl be reinvestigated and that other new fungistatic or fungicidal agents found useful on citrus be investigated for their possible acute and chronic toxicity in experimental laboratory animals. The need for retarding or preventing stem-end rot and other fungus diseases of citrus by means of chemical agents which do not present a health hazard to the producer or consumer is well illustrated by the studies on 2-aminopyridine and the present uncertain status of biphenyl. (See report on Toxicity of 2-aminopyridine).

Enzyme Systems in Citrus Fruits. A more comprehensive study should be made of the enzyme systems present in citrus fruits and their relationship to the biological changes which occur during the interval between harvesting and processing. Particular emphasis should be placed on learning in comprehensive detail the nature and mechanism of the changes that go on in fruit after harvest and while it is being held for processing, especially those changes which lead eventually to rapid breakdown. The enzymes involved in these reactions are still so imperfectly understood that practical procedures for enhancing the desirable changes and inhibiting the undesirable ones have been discovered mainly by trial and error; the potentialities of purposeful control may not yet have been more than touched. Research should be intensified until the key to this control is clearly in our hands.

Time-temperature Tolerance of Frozen Citrus Juice Concentrates. Study of the time-temperature tolerance of frozen citrus juice concentrates should be expanded to include lemon juice and grapefruit juice concentrates. A fundamental study of the chemistry and mechanism of cloud loss in frozen concentrates should be initiated. In the course of this work, any occurrence of the defect known as "cardboard" off-flavor should be exhaustively correlated with all the known conditions, in order to discover leads toward the explanation and elimination of this defect. It has become increasingly evident that the phenomenon of cloud loss cannot be explained or controlled solely on the basis of pectic enzymes and pectic changes in these products. Nor can the cloud stabilization achieved through heat

treatment be predicted on the basis of any presently known measurement such as the inactivation of pectic enzymes. A thorough study should be made of the physical and colloidal chemistry of cloud itself, and of the various enzymatic and non-enzymatic reactions possible in frozen concentrates which may contribute to, or actually initiate, the loss of cloud on storage. The objective of this work should be ultimately to develop new and accurate methods of predicting the minimum heat treatment needed to produce a given amount of cloud stability in different lots of orange juice and/or discover and develop methods, not involving heat treatment, which will produce stable cloud in frozen citrus concentrates. (See report on Time-Temperature Storage Studies of Frozen Orange Concentrate).

Compositional Studies on Fresh and Processed Citrus Juices. Work on the chemical composition of citrus juices should be expanded as rapidly as possible to include a comprehensive study of the identity and reactivities of all the important constituents present in orange, grapefruit and lemon juices. Particular emphasis should be placed on the determination of the chemical changes which take place in these constituents leading to the development of off-flavors, colors, and odors as a result of processing and storage. More rapid advancements in processing procedures for citrus products are possible if our knowledge of their composition and deterioration changes becomes more comprehensive and exact. For example, our newer knowledge of the presence of significant quantities of free amino acids in citrus juices may be important from the standpoint of nutritive value, as well as in identifying the causative agents and reactions of

deterioration. Work on the nitrogenous constituents should be expanded and accelerated to include a comprehensive quantitative as well as qualitative study of the amino acids in the important citrus juices, and particularly the nature of the chemical changes which they undergo during processing and storage. Work on the volatile flavoring constituents should be expanded to include a study of canned and stored lemon juice. Work on the flavonoids is now restricted to the identity of these constituents in lemon products. As results are accumulated and techniques developed, this study should be expanded to include other citrus juices. Work on the identity of the organic acids in citrus juices should be initiated, with particular reference to the part they play in flavor changes.

Microbiology of Frozen Citrus Concentrates. Work on the source of contamination, incidence and sanitary significance of coliform organisms in frozen concentrated orange juices should be expanded to include frozen lemon juice and concentrates. Work should be continued on the testing of various cultural media for the detection of coliforms in citrus products. Microbiological studies on frozen citrus products should be expanded to include a study of other organisms, such as Leuconostoc and Lactobacillus organisms, and the role they play in producing off-flavors in processed products. (See report on Microbiology of Frozen Orange Concentrates).

Effect of Pesticides on Flavor of Citrus Products. Since new insecticides and fungicides are constantly being proposed for use on citrus fruit

that is to be ultimately processed, current work to determine whether these pesticides cause any detectable off-flavor in orange and lemon products should be materially expanded and expedited. The study should be made more comprehensive, with respect both to the number of pesticides tested and as to the sensitivity of the tests for detecting these materials in the treated commodity. (See report on the Effect of Pesticides on Flavor of Orange Juice Products).

Physiological Action of Citrus Flavonoids. It is recommended that research on the identification, separation, and purification of the important citrus flavonoids, and study of their physiological effects on laboratory animals, should be increased because of the increasing evidence that the naturally occurring flavonoids in citrus fruits, as in other fruits and vegetables, exhibit physiological activity in a variety of ways. (See report on Identification of Flavonoid Constituents in Lemon Juice Products).

PROGRESS ON WORK UNDER WAY

Development of New and Improved Products Processes, and Equipment

CRANGE

New flavor-fortified Frozen Orange Superconcentrates BAIC

The developmental phase of the work on frozen orange superconcentrates prepared by adding cold pressed peel oil in place of "cut-back" juice has been essentially completed at the Pasadena Laboratory. Previously reported constant-temperature storage studies have shown: (1) that cloud stability at elevated temperatures increases as concentration of the stored product increased above 4-fold, and (2) that at the 6- and 7-fold levels of concentration, a degree of cloud stability is attained without heat treatment which is of the same order of magnitude as the cloud stability obtained in commercial 4-fold concentrate by the present methods of heat treatment.

Studies are now under way to determine the extent of additional cloud stability that may be attained by combining mild heat treatment with superconcentration, and to evaluate the possibility of producing stabilized orange superconcentrates capable of storage at room temperature or under moderate refrigeration (40° F.). A new type of storage study is being conducted with both heat-treated and unheated orange superconcentrates in which they are subjected to the cumulative fluctuating temperature patterns employed in the time-temperature storage studies. In this manner the comparative stability of orange superconcentrates versus commercial 4-fold concentrates will be obtained under time-temperature conditions

representative of those actually encountered during shipping, warehousing and retail marketing. This information is necessary for developing improved processed products which will more satisfactorily meet the requirements of the usual methods of handling such products.

A fundamental study has been initiated to determine the causes of increased cloud stability in high density superconcentrates. Although these studies are of a preliminary nature it has already been determined that much of the increased cloud stability in superconcentrates is due to the higher concentrations of sugar and citric acid occurring in these products. Conversely, it has been found that the addition of sugar and/or citric acid to commercial 4-fold concentrate will increase the cloud stability of this product in proportion to the amounts added. The manner in which these constituents contribute to cloud stabilization has not yet been determined.

In summary, the developmental phase of the work on frozen orange juice superconcentrates prepared by adding cold pressed peel oil has been completed. Studies are under way to determine the extent of additional cloud stability that may be attained by combining mild heat treatment with superconcentration. These studies will be continued during the coming year. In addition, a fundamental study has been initiated and will be continued at its present level to determine the causes of increased cloud stability in high density superconcentrates. (See Proposals for Committee Consideration).

Publications: Flavor fortified high density frozen citrus concentrates, R. G. Rice, G. J. Keller, R. J. McColloch, and E. A. Beavens, Abstracts

of Papers, 18A, 123rd Meeting of the American Chemical Society, Los Angeles, California, March 1953; Stabilization of frozen citrus concentrates by heat treatment, G. J. Keller, R. G. Rice and R. J. McColloch, Food Technology 7, Tech. Program and Abstracts 19, #57 (1953).

Heat Treatment of Citrus Juice Concentrates by Steam Injection BAIC

Studies have been undertaken at the Pasadena Laboratory on the application of direct steam injection heating for the rapid heat-processing of orange concentrates to effect maximum cloud stability with minimum flavor change. These studies included investigations of the time-temperature effects of steam injection heating, and the interrelationships of time and temperature of heating and concentration of the product at time of heating. The conditions studied included temperatures of 150°, 175° and 200° F. for 1 and 20 seconds holding times, applied to juices of 12°, 27°, 43° and 58° Brix concentration.

Results of these studies showed that concentration of the product to which a particular heat treatment was applied was an important variable having a marked effect on inactivation of pectinesterase and degree of cloud stability obtained. Application of heat at any of the higher juice concentrations brought about a greater inactivation of pectinesterase, and a greater increase in cloud stability than was obtained when single strength juices were heated. However, the greater the juice concentration and the higher the temperature used, the greater the possibility of flavor damage.

Under the conditions of these experiments, the effect of temperature on

cloud stability was greater than the effect of holding time. There was some indication that at certain "critical" temperatures, increased holding time may adversely affect cloud stability. Thus, the use of relatively mild temperatures for short holding times applied to concentrated juices will bring about similar cloud stabilities obtained by application of higher temperatures to single strength juices.

It was concluded that a high degree of cloud stability can be attained using direct steam injection heating without materially altering the flavor of the juice. This phase of the work is completed. (See Proposals for Committee Consideration).

Publications: Stabilization of frozen citrus concentrates by heat treatment. G. J. Keller, R. G. Rice and R. J. McColloch. Food Technology 7, Tech. Program and Abstracts 19, #57 (1953).

Stability of Orange Juice Powder BAIC

Work has been continued at the Western Regional Research Laboratory on procedures for preparing orange juice powder having increased storage stability. Most of the work during the past year has been confined to the preparation and storage of pure orange powder without additives. Laboratory drying procedures that have been developed for preparing orange powder with 40 percent added corn sirup solids were found to be satisfactory for pure orange powder except that somewhat lower pressures are required and the drying cycle is extended by about 30 minutes.

Comprehensive storage studies have been completed on orange powder containing 40 percent corn sirup solids. By the use of (1) in-package desiccation, (2) sorbitol to stabilize flavor components and (3) small amounts of sulfur dioxide in the powder, storage stability was increased to the extent that the powder was acceptable after 6 months of storage at 100° F. and had deteriorated only slightly after one year of storage at 70° F.

A commercial company has delivered 10,000 pounds of orange juice powder with added corn sirup solids to the Quartermaster Corps for field evaluation. This material was prepared by the procedure developed by the Western Regional Research Laboratory. According to reports received from the Quartermaster Corps, this powder has been entirely satisfactory. Another trial procurement order for 100 percent orange juice powder has recently been placed by the Quartermaster Corps with the same company.

Cooperative work by the Western Regional Research Laboratory with a commercial equipment manufacturer is under way to evaluate a continuous belt-type vacuum drier in an attempt to lower production costs inherent in the batch-type methods that have been used in preparing orange powder. These experiments, while not yet complete, indicate that this drier may be suitable for continuously producing orange juice powder on a large scale.

A preliminary study was made of the costs associated with the production and distribution of orange juice powder. The cost picture is favorable for the product in its intended uses. Exact processing cost estimates can not be made at present, however, because the commercial

process has not yet been fully developed.

Comprehensive stability studies on 100 percent orange powder, using improved in-package desiccation procedures, are under way at the present time.

GRAPEFRUIT

New Flavor-fortified Frozen Grapefruit Superconcentrates

BAIC

Time-temperature storage studies under conditions of cumulative, fluctuating temperature patterns simulating those which may actually occur in commercial shipping, warehousing and retail distribution are being continued on grapefruit superconcentrates at the Pasadena Laboratory.

Exploratory production and storage tests have been conducted on grapefruit juice concentrates stabilized for room temperature (70° F.) storage by a combination of superconcentration and mild heat treatment. Results indicate that the most serious problem in these products is rapid and excessive browning. As might be expected, the rate of browning increases with increasing concentration of the product. So far no satisfactory method to prevent or inhibit the browning has been found.

Storage studies at other temperatures will be continued during the coming year. (See Proposals for Committee Consideration).

Publications: Flavor fortified high density frozen citrus concentrates, R. G. Rice, G. J. Keller, R. J. McColloch and E. A. Beavens, Abstracts of Papers, 18A, 123rd Meeting of the American Chemical Society, Los Angeles, California, March 1953; Stabilization of frozen citrus

concentrates by heat treatment, G. J. Keller, R. G. Rice and R. J. McCulloch, Food Technology 7, Tech. Program and Abstracts 19, #57 (1953).